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Kinetic resolution and deracemisation of racemic amines using a reductive aminase

Godwin A. Aleku, Juan Mangas-Sanchez, Joan Citoler, Scott P. France, Sarah L. Montgomery, Rachel S. Heath, Matthew P. Thompson and Nicholas J. Turner. *

Abstract: The NADP(H)-dependent reductive aminase from *Aspergillus oryzae* (*Asp*RedAm) has been combined with an NADPH oxidase (NOX) to develop a redox system that recycles the co-factor. The *Asp*RedAm-NOX system has been applied initially for the kinetic resolution of a variety of racemic secondary and primary amines to yield (*S*)-configured amines with enantiomeric excess (*e.e.*) up to 99%. Addition of ammonia borane to this system enabled the efficient deracemisation of racemic amines, including the pharmaceutical drug rasagiline and the natural product salsolidine, with conversions of up to >98% and *e.e.*'s >99% *e.e*. Furthermore, using the variant *Asp*RedAm W210A it was possible to generate the opposite (*R*)-enantiomers with efficiency comparable to, or even better than, the wild-type *Asp*RedAm.

A significant proportion of small molecule therapeutics contain one or more chiral amine functional groups and indeed a recent evaluation of novel drug approvals highlights the prominence of these moieties as building blocks of pharmaceuticals.[1] Consequently, a number of amine-forming synthetic methods, utilising transition metal catalysis, organocatalysis or biocatalysis, have been developed.[2] Enzymatic transformations are often highly chemo-, regio- and stereoselective, and operate under mild and environmentally-friendly conditions.[3] As a result, preparative biocatalytic routes to chiral amines employing lipases,[4] amine oxidases,[5] amine transaminases (ATAs),[2c, 6] ammonia lyases,[7] amine dehydrogenases (AmDHs)[8] and imine reductases (IREDs),[9] or engineered variants of these biocatalysts, have received considerable attention.

Production of enantiopure amines can be achieved *via* biocatalytic kinetic resolution (KR) orderacemisation (DR) of racemic amines. Although KRs using lipases and transaminases are widely used, they possess the inherent limitation of a maximum yield of 50%. This issue may be addressed by the conversion of KRs to deracemisation methods such *as* dynamic kinetic resolutions (DKR) in which an additional biocatalyst or chemical reagent is added to racemise *in situ* the unreacted enantiomer.[10,11] Another important deracemisation approach involves the interconversion of the two enantiomers of the amine, usually *via* the corresponding imine. For example, the deracemisation of racemic amines employing amine oxidases has been achieved with repeated cycles of enzyme-catalysed enantioselective amine oxidation and non-selective chemical imine reduction using ammonia borane ($NH_3.BH_3$)[5b] or sodium borohydride ($NaBH_4$)[5c], resulting in yields that are comparable to asymmetric synthetic methods (Figure 1a).

Recently we reported the discovery of an NADPH-dependent reductive aminase (RedAm) from *Aspergillus oryzae* (*Asp*RedAm) and demonstrated the application of this biocatalyst in the synthesis of mostly (*R*)-configured chiral amines from the corresponding ketones.[12] For certain amine/ketone combinations, *Asp*RedAm is able to catalyse reductive amination with ratios as low as 1:1. RedAms are part of the IRED enzyme family and thus can also catalyse the reverse reaction, namely the oxidation of amines to imines at the expense of $NADP^+$. We envisaged exploiting this reverse activity as a means of gaining access to the opposite (*S*)-enantiomers using *Asp*RedAm. Indeed IREDs have previously been reported to catalyse the kinetic resolution of racemic amines although with low efficiency (9% conv; 10% *e.e.*).[13] Herein we report the application of *Asp*RedAm for both the kinetic resolution and deracemisation of racemic amines (Figure 1b).

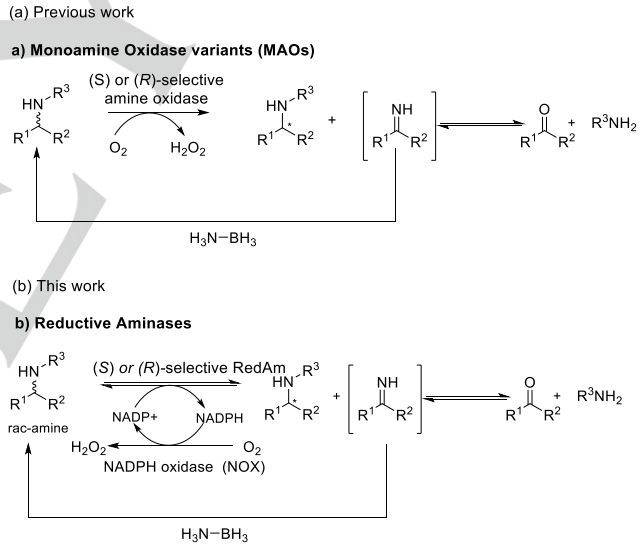

Figure 1. Biocatalytic deracemisation of racemic amines *via* (a) monoamine oxidase (MAO)-catalysed route and b) *Aspergillus* reductive aminase (*Asp*RedAm)-catalysed route.

In order to assess the potential for the application of *Asp*RedAm in the kinetic resolution (KR) of racemic amines, we initially examined the oxidative deamination activity towards a panel of amine substrates comprising cyclic and acyclic 1°, 2° and 3° amines. Initial reaction rates were measured at pH 10.5 and revealed the preference of *Asp*RedAm for secondary amines, with the highest specific activity of 6.5 U mg^{-1} observed towards 1-methyl-1,2,3,4-tetrahydroisoquinoline **1** (Figure 2).

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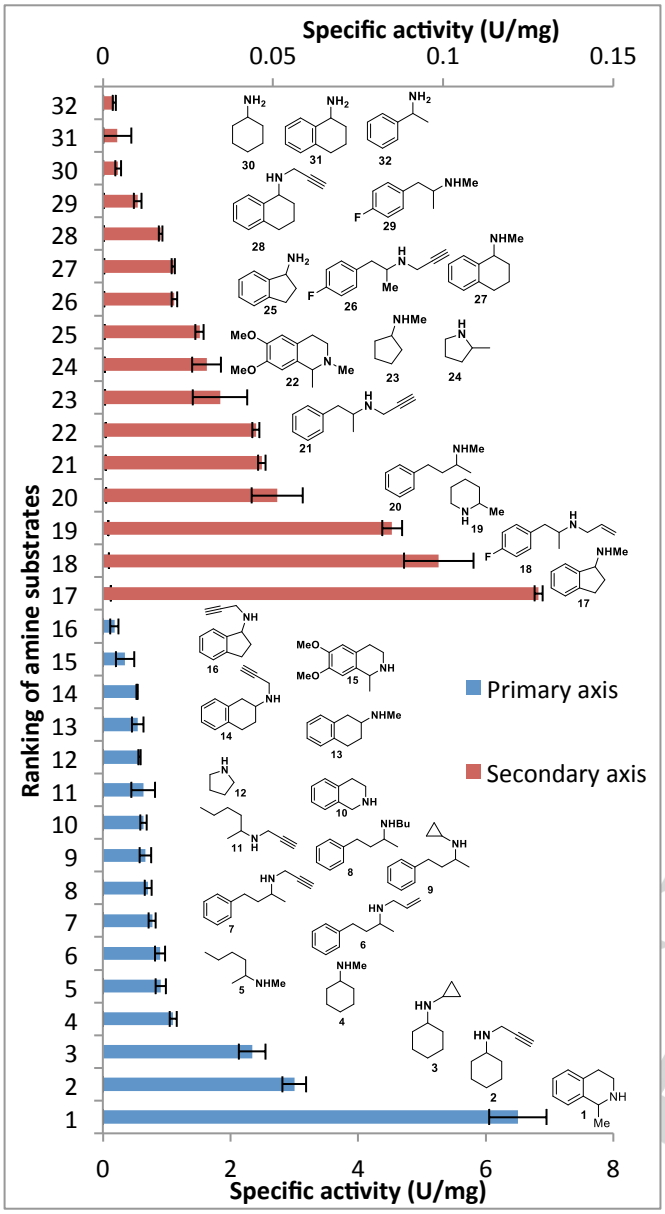


Figure 2. Substrate specificity of *Asp*RedAm in the NADP$^+$-dependent oxidative deamination of amines **1**-**32**. Two y-axes have been used to allow representation of high (blue) and moderate (red) specific activities. Activities were measured in triplicate and the error bars represent standard deviation from the mean specific activity.

In general, the relative specific activities for the oxidation of amine substrates follow similar trends to the activities observed in the synthetic direction, as previously reported.[12] For example, *Asp*RedAm displayed higher activities of 0.9-2.3 U mg^{-1} for *N*-alkyl-cyclohexylamines (**2**, **3** and **4**) whereas the enzyme's activity towards cyclohexylamine **30** (0.010 U mg^{-1}) and most primary amines (0.003 – 0.028 U mg^{-1}) were up to two orders of magnitude lower. Interestingly, the type of *N*-alkyl substituent appears to exert a significant effect on the catalytic rates, with *Asp*RedAm displaying higher specific activities in the oxidation of *N*-allylamine, *N*-propargylamine, *N*-butylamine and *N*-cyclopropylamine derivatives than with *N*-methyl derivatives. For example, the initial rates for the oxidations of **6**-**9** (0.90 – 0.65 U mg^{-1}) were up to an order of magnitude greater than the oxidation of **20** (0.05 U mg^{-1}) even though these compounds differ only by the *N*-alkyl substituents.

Based upon this initial assessment we then applied *Asp*RedAm for the KR of racemic amines *N*-(4-phenylbutan-2-yl)butan-1-amine **8** and rasagiline **16**. The enantioselective deamination of these racemic amines led to the accumulation of the (*S*)-enantiomer in excellent *e.e.* of >99% and conversion of 49% when a stoichiometric amount of NADP$^+$ was used. The deamination was also observed to be highly regioselective, *e.g.* HPLC and GC analysis of biotransformations for the deamination of racemic amines **8** and **16** and **28** confirmed that hydride abstraction occurred at the stereogenic carbon atom rather than from the *N*-alkyl group (Supporting Information, Section 1.3).

We next addressed the issue of *in situ* co-factor recycling and evaluated the incorporation of an NADPH oxidase, an enzyme which mediates the transfer of electrons from NADPH to molecular oxygen, generating hydrogen peroxide as the by-product.[14] Using two model amine substrates **8** and 1-aminoindane **25**, the optimal reaction pH and buffer conditions were determined. The *Asp*RedAm-NOX coupled system tolerated pH values between 8.0 and 11.0, however the best conversions (up to 50%) were achieved at pH values between 10.0 and 10.5 (Supporting Information Section 1.1). Control experiments featuring all reaction conditions including NOX but lacking either *Asp*RedAm or the cofactor did not result in any conversion.

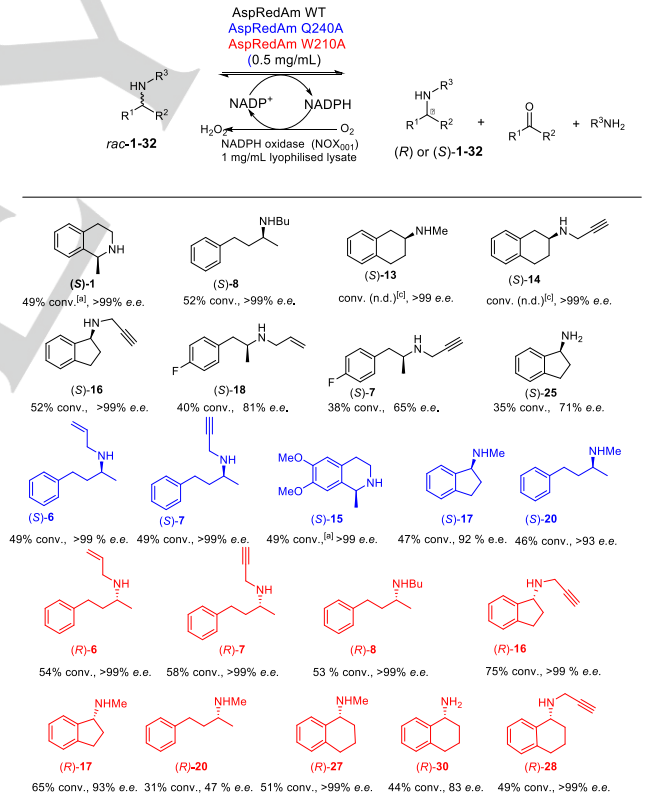


Figure 3. Biotransformation results of *Asp*RedAm-catalysed kinetic resolution of racemic amines employing an NADPH oxidase to recycle the cofactor. Resolution of the racemic amines were achieved using either AspRedAm wild-type (WT) enzyme (black), *Asp*RedAm Q240A (blue) or AspRedAm W210A (red). Conversions were determined by HPLC or GC-FID analysis. Reaction conditions: racemic amine (5 mM), *Asp*RedAm (0.5 mg mL^{-1}), NADP$^+$ (0.3 mM) and lyophilised lysate powder of prozomix NADPH oxidase Pro-NOX001, (1 mg mL^{-1}). Reactions were run at pH 10.0 (100 mM glycine-NaOH buffer) unless stated otherwise. Reactions were incubated at 25 °C with 250 rpm shaking for 24 h. [a] Reactions were run at pH 8.0 (100mM Tris-HCl buffer). [c] Conversion not determined as only negligible amount of the ketone

formed from the *Asp*RedAm-catalysed deamination was detected. This may be due to further sponstaneous oxidation of the resulting ketone.

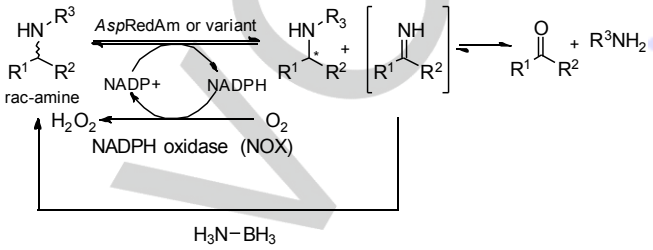
The *Asp*RedAm-NOX system was applied to the KR of a panel of racemic secondary and primary amines (Figure 3), affording >99% *e.e.* in most cases for secondary amines, and up to 83% *e.e*. for primary amines. The KR of *N*-allyl-4-phenylbutan-2-amine **6** and 4-phenyl-*N*-(prop-2-yn-1-yl)butan-2-amine **7** resulted in high conversion to ketone (up to 80%) due to the poor enantioselectivity of the wild-type enzyme towards these substrates. To address this issue, we employed a variant of *Asp*RedAm (Q240A) which displays improved (*R*)-selectivity, affording 49% conversion and >99 *e.e.* for **6** and **7**. Interestingly, the Q240A variant also showed improved efficiency towards a range of substrates (Figure 3, Supporting Information Section 1.2, Table S1), affording higher conversions and *e.e.*'s than the wild type enzyme.

Encouraged by the efficiency and improved selectivity of the Q240A variant, we attempted to further expand the product profile of the *Asp*RedAm-NOX KR system. Previously, we reported a variant of *Asp*RedAm W210A which in several cases displayed opposite enantioselectivity when compared to the wild-type enzyme.[12] The (*S*)-selective *Asp*RedAm W210A yielded the corresponding (*R*)-configured products in good to excellent *e.e.s* (Figure 3, Supporting Information Section 1.2, Table S2). *Asp*RedAm W210A catalysed KR allows access to novel (*R*)-configured amine products, which are difficult to access with MAO-N variants,[5d] and therefore expands the substrate scope of (*S*)- selective KR biocatalysts.

Finally we explored the development of an *Asp*RedAm-NOX based deracemisation system in order to achieve yields of up to 100%. Previously we and others have shown that deracemisation of amines with monamine oxidases[5] and PALs[7b] can be achieved by the addition of a mild chemical reducing agent such as ammonia borane ($NH_3.BH_3$) to reduce *in situ* the corresponding imine. Repeated cycles of selective oxidation of the reactive enantiomer catalysed by *Asp*RedAm, and non-asymmetric imine reduction by $NH_3.BH_3$, was envisaged to lead to the complete deracemisation of racemic amines, provided that the co-factor was also continuously recycled by NOX. This cascade was evaluated by performing biotransformations for the deracemisation of **1** and **8** at different pHs (7.0-11.0). Interestingly, conversions of >95% were achieved for **1** and **8** at pH values of 8.0 and 10.0 respectively using 4 equiv. of $NH_3.BH_3$, hence subsequent biotransformations were performed at pH 8.0 and pH 10.0 for cyclic and acyclic amines respectively (Table 1). Control biotransformations were performed and featured all reaction components and conditions but lacking either *Asp*RedAm or $NADP^+$; in both cases the racemic amine starting materials remained unconverted.

By using the one-pot *Asp*RedAm-NOX-$NH_3.BH_3$ system, the deracemisation of a number of racemic cyclic and acyclic amines was achieved with conversion of up >98% and up to >99% *e.e* (Table 1). In general the formation of alcohol side product (resulting from imine hydrolysis and subsequent reduction of the ketone by $NH_3.BH_3$) was not observed for most of the substrates screened. This observation contrasts with that found with the MAO-N based deracemisation system[15] and is presumably a consequence of the ability of *Asp*RedAm to recycle the ketone back to the amine via the imine. However, for the deracemisation of rasagiline **16**, both indanone (up to 18%) and indanol (up to 34 %) were detected in amounts that depended on the number of equivalents of $NH_3.BH_3$ used. This limitation may be addressed by the use of other chemical reducing agents for imine reduction as recently reported.[5c] To demonstrate preparative scale application, we performed 100 mg scale biotransformations for the deracemisation of racemic amines **1** and **16** (20 mM) using 4 equiv. and 10 equiv. of $NH_3.BH_3$ respectively. Deracemisation of racemic **1** yielded (*S*)-**1** (78% isolated yield and 93% *e.e.*) after 24 h, while (*S*)-**16** was obtained after 48 h (64% isolated yield and >98% *e.e.*) from deracemisation of racemic amine **16**.

Table 1. Biotransformation results for the deracemisation of racemic amines using the *Asp*RedAm-NOX-$NH_3.BH_3$ cascade.



Entry	Racemic amine	$NH_3.BH_3$ equiv.	*Asp*RedAm variant	Conv. [%]	*e.e.* [%] (*R* or *S*)
1	**1**[a]	2	*Asp*RedAm wt	>98	>98 (*S*)
2	**6**	4	*Asp*RedAm Q240A	>97	96 (*S*)
3	**6**	4	*Asp*RedAm W210A	>97	94 (*R*)
4	**8**	4	*Asp*RedAm wt	>98	>99 (*S*)
5	**8**	4	*Asp*RedAm W210A	>98	>99 (*R*)
6	**15**[a]	10	*Asp*RedAm Q240A	>97	>99 (*S*)
7	**16**	4	*Asp*RedAm wt	74[b]	93 (*S*)[b]
8	**20**	10	*Asp*RedAm Q240A	>97	>99 (*S*)

Reactions were run at pH 10.0 (100 mM glycine-NaOH buffer) unless stated otherwise. Reactions were incubated at 25°C with 250 rpm shaking for 24 h. [a] Reactions were run at pH 8.0 (100mM Tris-HCl buffer). [b] 18% and 34% indanol were detected with 10 equiv. and 5 equiv. of $NH_3.BH_3$ respectively. wt = wild-type. Conv. = conversion.

In summary we have shown that the reductive aminase from *Aspergillus oryzae* (*Asp*RedAm) can be employed for both the kinetic resolution and deracemisation of a range of cyclic and acyclic racemic amines. A key element of both systems is the use of an NADPH oxidase for *in situ* co-factor recycling of $NADP^+$. The *Asp*RedAm-NOX-$NH_3.BH_3$ system enables deracemisation of a variety of cyclic and acyclic racemic amines to provide access to enantiomerically pure (*S*)-configured amine products. Furthermore, switching to the rationally engineered single point variant of *Asp*RedAm W210A enables access to (*R*)-configured products.

Experimental Section

Details of experimental procedures are described in Supporting Information Section 2.

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Keywords: Biocatalysis • Chiral amines • Deracemisation • Kinetic resolution • Reductive aminase

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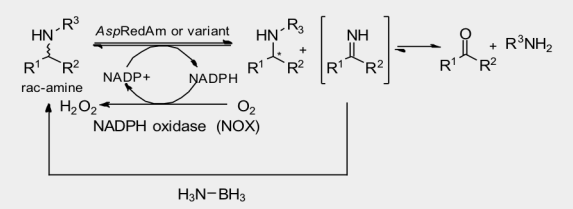

The reductive aminase from *Aspergillus oryzae* (AspRedAm) has been combined with NADPH oxidase (NOX) to enable the kinetic resolution of a variety of racemic amines yielding (*S*)-configured products. The corresponding (*R*)-enantiomers were obtained by the use of a single amino acid variant AspRedAm W210A. Furthermore, *Asp*RedAm-NOX-NH_3.BH_3 cascade was constructed to allow the efficient deracemisation of racemic amines.

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